Volume 16 Number 19 1988 Nucleic Acids Research

Polymerase chain reaction amplification of rhinovirus nucleic acids from clinical material

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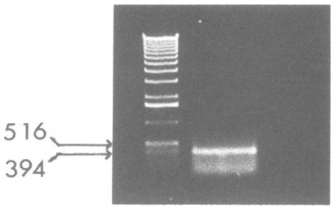
Submitted August 30, 1988

Human rhinoviruses (HRVs) are the major cause of the common cold and are important pathogens. Many HRVs grow poorly and so are difficult to study and since they have an RNA genome and are highly mutable, propagation in culture is also likely to lead to the accumulation of mutations. Potentially, the polymerase chain reaction (PCR) can circumvent these problems by amplifying sufficient material from clinical samples to analyse directly (1). Prior to PCR reverse transcription needs to be performed to copy the RNA genome into cDNA. Further complications are the nature of clinical samples, the presence of nucleases and the relatively low levels of the viruses, all of which hinder RNA isolation and subsequent enzymic manipulation. Here we describe a method which enables HRV nucleic acid to be extracted from clinical material and amplified by PCR to give levels of DNA sufficient for further study.

Nasal washings were obtained from an individual infected with HRV-2. To a 100ul aliquot (with a titre of $10^{5.5}$ $TCID_{50}ml^{-}$) was added tRNA (0.5µg) to act as a carrier and Vanadyl ribonucleoside complexes (VRC, 10mM) as an RNase inhibitor. The sample was extracted with phenol/water (100µl) and after centrifugation the phenol layer and interphase were re-extracted with water (100µl). The pooled aqueous layers were re-extracted 4x with phenol/water and 2x with chloroform. RNA was collected by precipitation and centrifugation and was reverse transcribed in a solution (40µl) containing 50mM Tris-HCl pH 8.3, 75mM KCl, 10mM DTT, 3mM $MgCl_2$, 0.5mM dNTPs, RNaseGuard (40 U), primer OL27 (0.1nmole) and MMLV reverse transcriptase (200 U) at 37°C for 1 hr. 20 cycles of PCR were performed (95°C for 1 min., 55°C for 2 min. and 70°C for 4 min.) on a 5µl aliquot of the product in 100µl of 10mM Tris-HCl pH 8.3, 50mM KCl, 2.5mM $MgCl_2$, 0.2mM dNTPs, OL26 and OL27 primers (0.1nmole) and 2.5U Taq polymerase (Anglia Biotech). Further polymerase was added after 10 cycles. Gel electrophoresis of the product (Figure) revealed the presence of amplified DNA approximately 380 nucleotides in length. This corresponds to the distance between the primers which are complementary to HRV-2 nucleic acid at positions 163-178 (-ve,OL26) and 531-546 (+ve,OL27)(2). The procedure has been repeated for several HRVs and works reproducibly, critical features being the inclusion of tRNA and VRC during RNA isolation. The amplification of HRV nucleic acid from clinical material has implications for viral diagnosis methods as well as enabling the future detailed analysis of strains of known pathogenicity.



Acknowledgement This work was supported by the Medical Research Council.

References:1. Saiki et al (1988) Science 239, 487-494. 2. Skern et al (1985) Nucleic Acids Research 13, 2111-26.